亜鉛サイクレン錯体を吸着させた アガロース含有マイクロチップの開発による チオール基含有化合物の選択的分離精製法

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A novel thiol-affinity micropipette tip method usingzinc(II)–cyclen-attached agarose beads for enrichment ofcysteine-containing molecules

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ABSTRACT: Cysteine-containing biomolecules are attractive targets in the study of thiol biology. Here we intro-duce a novel method for the selective enrichment of thiol-containing molecules using a thiol-capture zinc(II) complex of 1,4,7,10-tetraazacyclododecane (Zn^{2+}–cyclen). Recognition of N-acetylcysteine amide by Zn^{2+}–cyclen has been studied by potentiometric pH titration, revealing formation of a 1:1 thiolate-bound Zn^{2+}–cyclen complex with a large thiolate-affinity constant of $10^{6.2}M^{-1}$at 25℃ and I = 0.10 M(NaCl). The Zn^{2+}-bound thiolate anion is unexpectedly stable in aqueous solution at pH 7.8 under atmospheric conditions for a few days. These findings have contributed to the development of a convenient method for separation of thiol compounds by using a micropipette tip. A 200 μL micropipette tip containing 10 μL of hydrophilic cross-linked agarose beads attached to Zn^{2+}–cyclen moieties was prepared. All steps for thiol-affinity separation (binding, washing, and eluting) are conducted using aqueous buffers at room temperature. The entire separation protocol requires less than 15 min per sample. We demonstrate practical example separations of cysteine-containing molecules. This micropipette tip method would be used preferentially as an alternative to existing tools for reliable enrichment of thiol-containing molecules.

抄録　システイン残基であるチオール基は、生体内で重要な機能を有しており研究の対象として注目されているが、チオール基を選択的、かつ可逆的に捕捉する亜鉛サイクレン錯体を開発している。この錯体をアガロースゲルに吸着させマイクロチップに詰めてチオール含有分子の分離精製法を開発し、従来の方法よりも簡単かつ短時間でシステイン含有ペプチドのみをシステイン含有ペプチドを添加したβ－カゼイン消化物サンプルから単離することができた。

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